

Reprinted from

**Canadian
Journal of
Fisheries and
Aquatic
Sciences**

Réimpression du

**Journal
canadien des
sciences
halieutiques et
aquatiques**

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Stored at 4°C**

A. H. EL-SHAARAWI AND M. A. NEILSON

Volume 41 • Number 6 • 1984

Pages 985-988

Canada



Fisheries
and Oceans

Pêches
et Océans

Changes in Nutrient Levels of Lake Water Stored at 4°C

A. H. El-Shaarawi

Department of Environment, National Water Research Institute, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

and M. A. Neilson

Department of Environment, Inland Waters Directorate, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

El-Shaarawi, A. H., and M. A. Neilson. 1984. Changes in nutrient levels of lake water stored at 4°C. *Can. J. Fish. Aquat. Sci.* 41: 985-988.

Water samples were collected on Lake Ontario during April and November, filtered (0.45 µm), and immediately analyzed onboard ship for the nutrients soluble reactive phosphorus, nitrate-plus-nitrite, and ammonia. Replicates were stored in glass bottles at 4°C and reanalyzed within 8 d. Statistical analysis showed that soluble reactive phosphorus decreased by 11 and 13% and nitrate-plus-nitrite by 7 and 6%, whereas ammonia increased by 75% on one cruise and decreased by 37% on the other.

Des échantillons d'eau prélevés en avril et novembre dans le lac Ontario et filtrés (0,45 µm) ont été immédiatement analysés à bord du navire pour le dosage des matières nutritives : phosphore réactif soluble, nitrate et nitrite et ammoniac. Des doubles ont été conservés à 4°C dans des bouteilles de verre et analysés moins de 8 jours après. Une analyse statistique a montré que les teneurs en phosphore réactif soluble avaient diminué de 11 et 13% et celles de nitrate et nitrite de 7 et 6% tandis que la teneur en ammoniac s'était accrue de 75% lors d'une mission et réduite de 37% lors de l'autre mission.

Received May 3, 1983

Accepted February 27, 1984

Reçu le 3 mai 1983

Accepté le 27 février 1984

Considerable research (Eichholz et al. 1965; Thayer 1970; Bowditch et al. 1976; Macdonald et al. 1980) has been conducted to study the effect of preservation and storage on the chemical characteristics of water samples. Soluble reactive phosphorus (SRP), nitrate-plus-nitrite ($\text{NO}_3 + \text{NO}_2$), and ammonia (NH_3) have received particular attention (Murphy and Riley 1956; Gilmartin 1967; Jenkins 1968; Howe and Holley 1969; Thayer 1970; Armstrong 1972; Degobbi 1973; Klingaman and Nelson 1976). Stored without treatment, the concentration of these nutrients can undergo significant change. These changes are rapid, occurring within 30 min of sampling (Collier and Marvin 1953). There is little agreement as to the optimal preservation technique for these nutrients. To stabilize the SRP content of water samples, Collier and Marvin (1953) recommended quick-freezing and storage at subzero temperatures. Strickland and Parsons (1968) advised analysis within 2 h of sampling, but agreed that freezing would stabilize the sample for several months. Gilmartin (1967) and Philbert (1973), in studying the effect of freezing on nutrient concentrations, found decreased SRP levels in the thawed samples. Various sources suggested mercuric chloride as an effective preservative in conjunction with either refrigeration (Environmental Protection Agency 1971; Klingaman and Nelson 1976) or freezing (Jenkins 1968; American Public Health Association 1971). However, Henriksen (1969) found that in the presence of mercuric chloride, SRP levels increased. Bowditch et al. (1976) determined the most acceptable preservation technique to be storage in glass bottles at 4°C, yet Murphy and Riley (1956) and Klingaman and Nelson (1976) reported large changes in SRP concentrations using this technique. Murphy and Riley (1956) considered storing sam-

ples at 20°C, in the dark, with the addition of various preservatives (sodium fluoride, chloroform, aluminum hydroxide, and thorium carbonate). Their conclusion, only to be refuted by Jenkins (1968), was that chloroform (0.7% v/v) worked best.

To preserve nitrogen samples, Bowditch et al. (1976) recommended storage at 4°C, but Klingaman and Nelson (1976) and Thayer (1970) found that large concentration changes resulted. Jenkins (1968) advised the addition of mercuric chloride, prior to refrigeration, for short-term storage of NH_3 , NO_3 , and NO_2 and, except for NO_2 samples, sulphuric acid for long-term storage. While this met with acceptance from Howe and Holley (1969) and the American Public Health Association (1971), it had previously been found to be inadequate for stabilizing NH_3 (Kreps 1934; Cooper 1937; Redfield and Keys 1938). Freezing was suggested as an alternative (Marvin and Proctor 1965; Klingaman and Nelson 1976), but Degobbi (1973) and Newell (1967) reported concentration changes with increased variability attributed to the rupturing of plant and animal cells during freezing and thawing. Phenol was found to stabilize NH_3 concentrations for up to 2 wk (Degobbi 1973).

The Water Quality Branch, Ontario Region, conducts a wide variety of studies requiring measurement of the phosphorus and nitrogen levels in water. In light of the inconclusiveness of the aforementioned studies, the analysis of SRP, $\text{NO}_3 + \text{NO}_2$, and NH_3 are performed, whenever possible, in the field at the time of sampling. However, as this is not always convenient, samples are sometimes collected and stored at 4°C. A comparative study was initiated in 1982 to determine if changes occur in the concentration of these nutrients as a result of storage. The objective of our study was to determine if there were real

TABLE 1. Detection limits, number (and percentage) of samples reported below these levels, and medians of shipboard and stored (laboratory) sample analyses (all concentrations are reported as mg/L).

Nutrient	Detection limit (dl)	No. of samples < dl (%)				Median			
		Apr. 26-30		Nov. 19-24		Apr. 26-30		Nov. 19-24	
		Ship	Lab.	Ship	Lab.	Ship	Lab.	Ship	Lab.
SRP	0.0002	0(0)	5(1)	0(0)	0(0)	0.0047	0.0041	0.0046	0.0040
NO ₃ + NO ₂	0.005	0(0)	0(0)	0(0)	0(0)	0.365	0.342	0.358	0.336
NH ₃	0.001	50(13)	12(3)	23(6)	107(29)	0.002	0.003	0.004	0.003

changes in the concentration of SRP, NO₃ + NO₂, and NH₃ as a result of storage and then their magnitude and confidence intervals.

Methods

In 1982, during the April 26-30 and November 15-19 Lake Ontario surveillance cruises, samples were collected for the analysis of these nutrients. Sampling was conducted at 94 stations on Lake Ontario at depths of 1, 10, 25, bottom-10 m, and bottom-2 m during isothermal conditions and at 1 m, 1 m above and below thermocline, bottom-10 m, and bottom-2 m during stratified conditions. Samples from each depth were filtered through 0.45-μm membrane filters and then split into two sets of replicates and stored in 125-mL glass bottles with plastic snap-on caps. One set was refrigerated (4°C) for future analysis; the other underwent immediate analysis in the shipboard laboratory for SRP, NO₃ + NO₂, and NH₃. During the April 26-30 and November 15-19 cruises, 374 and 370 samples, respectively, were collected. Replicate analyses were conducted May 4-5 and November 24-25 following each cruise.

SRP was measured using an automated colorimetric stannous chloride method, NO₃ + NO₂ was determined by an automated cadmium reduction, and NH₃ was determined by an automated colorimetric phenate procedure (Anonymous 1979). All analyses were performed on identical equipment in the laboratory and onboard ship by the same technicians.

The statistics were performed using the sign test with continuity correction and confidence intervals for medians as outlined by Lehmann (1975, Chap. 4). When either the before and after storage measurements were equal, or both were below the detection limit, the samples were ignored in the calculations, since no information about the effect of storage was provided.

Results and Discussion

The detection limits for the three nutrients and the number and percentage of samples reported below these levels are listed in Table 1. Also given are the median concentrations of the shipboard and stored sample analyses (x_i and y_i , respectively).

Plots of the empirical cumulative distribution functions (CDF) of the differences between the shipboard and stored sample analyses for the April cruise are presented in Fig. 1. The CDF for obtaining negative differences (i.e. storage results are higher than shipboard results) are 0.22, 0, and 0.64 for SRP (Fig. 1a), NO₃ + NO₂ (Fig. 1b), and NH₃ (Fig. 1c), respectively. These values strongly indicate that the distribution is not symmetric about zero and that storage results in (1) the reduction of the concentrations of SRP and NO₃ + NO₂ and (2) the increase of the concentration of NH₃. Also shown on each

graph is the estimated median of the distribution, which is larger than zero for SRP and NO₃ + NO₂ and smaller than zero for NH₃. The results for the November cruise are shown in Fig. 2. The CDF for obtaining negative differences are 0.27, 0, and 0.28 for SRP, NO₃ + NO₂, and NH₃, respectively. Also, the medians of the three nutrients are larger than zero (Fig. 2). From this it appears that the results for SRP and NO₃ + NO₂ are consistent for the two cruises, while the NH₃ results indicate an opposite storage effect.

Results of application of the statistical analysis to the nutrient data are presented in Table 2. The values of the sign test are highly significant ($P < 0.01$) indicating (1) for the April cruise, storage resulted in increasing the concentration of NH₃ and reducing the concentration of the other two nutrients and (2) for the November cruise, storage resulted in reducing the concentration of the three nutrients. The estimate of the median of the differences between the ship and laboratory analyses and the limits of the 95% confidence interval for the median are also given in the table. Note that the derivation of the confidence limits requires that the distribution of the differences be symmetric about the median. One way of checking symmetry is to compare the difference between the median and the first quartile with the difference between the third quartile and the median. If these are equal, the distribution is symmetrical. For the data used here, the assumption of symmetry appears to be valid.

The final column of the table lists the percent change in concentration resulting from storage, calculated as

$$\% \text{ change} = \frac{\text{median of the difference } (x_i - y_i)}{\text{median of shipboard analysis } (x_i)} \times 100.$$

It appears that the percent change was constant for SRP and NO₃ + NO₂ for the two cruises, but substantially different for NH₃. This might be due to a change in the concentration of the nutrients in the samples, i.e. the November concentration of NH₃ was double that of the April cruise, while SRP and NO₃ + NO₂ concentrations remained relatively constant.

The loss of NO₃ + NO₂ and subsequent increase in NH₃ concentrations on the first cruise may be indicative of the dominant bacterial species present in the water at that time, i.e. nitrifying bacteria were more sensitive to low temperatures than were those responsible for ammonification (Klingaman and Nelson 1976). Since the levels of both nutrients decreased on the November cruise, there must be an alternate explanation, such as algal uptake or adsorption. Likewise, decreased SRP concentrations may be a result of phosphate utilization by a developing bacterial population (Gilmartin 1967) or adsorption onto detritus.

In conclusion, storage of unpreserved water samples in glass

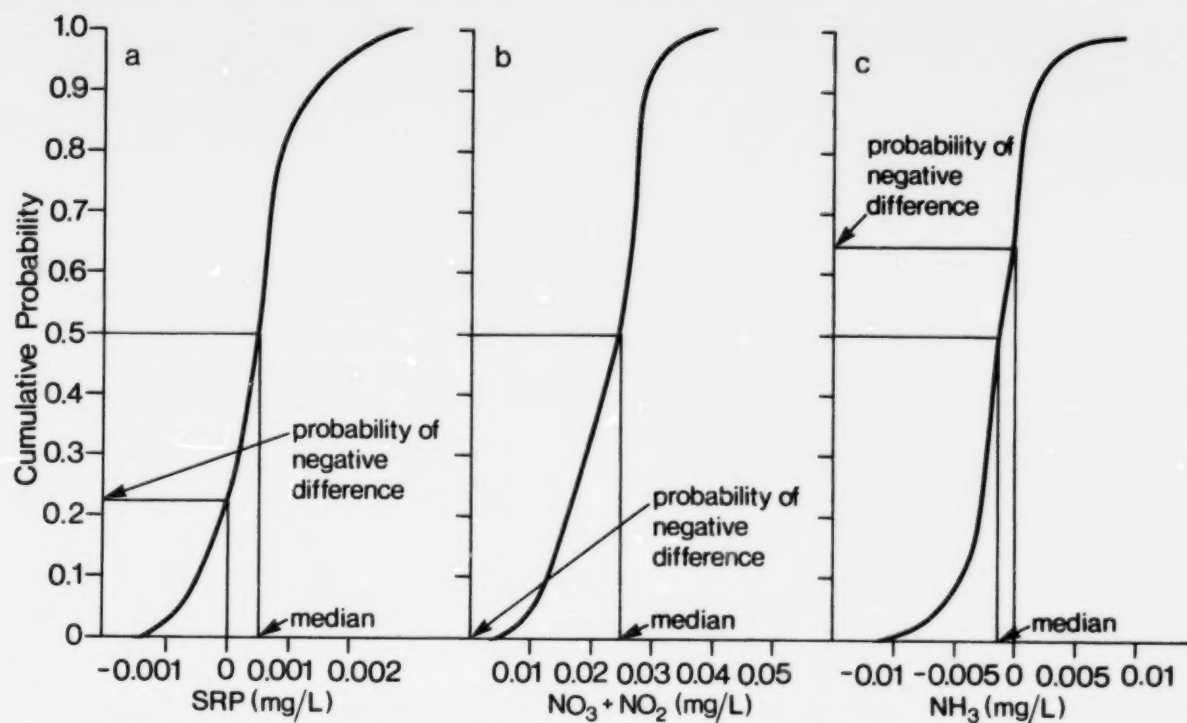


FIG. 1. Empirical cumulative distribution functions for the differences between the shipboard and stored sample analyses (April 26-30).

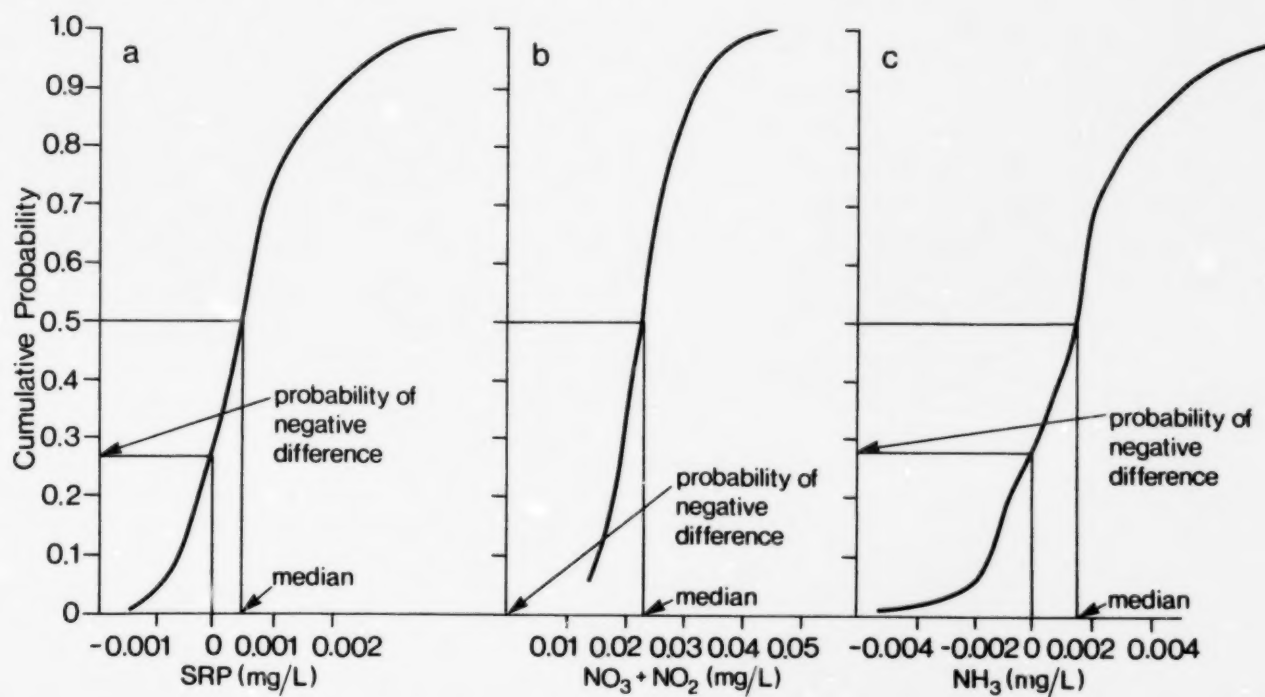


FIG. 2. Empirical cumulative distribution functions for the differences between the shipboard and stored sample analyses (November 19-24).

TABLE 2. Summary of the statistical analyses of the three nutrients (*Significant at the 1% level).

Cruise	Nutrient	No. of differences			Sign test	Median of differences	95% confidence interval		% change
		-ve	+ve	Total			Lower limit	Upper limit	
Apr. 26-30	SRP	70	288	358	11.47*	0.0005	0.0004	0.0006	11
	NO ₃ + NO ₂	1	373	374	19.18*	0.025	0.024	0.027	7
	NH ₃	192	108	300	-4.91*	-0.0015	-0.0020	-0.0009	-75
Nov. 19-24	SRP	94	257	351	8.65*	0.0005	0.0004	0.0006	11
	NO ₃ + NO ₂	0	370	370	19.18*	0.023	0.022	0.024	6
	NH ₃	64	238	302	9.96*	0.0015	0.001	0.002	37

bottles at 4°C has been found to be insufficient for the stabilization of SRP, NO₃ + NO₂, and NH₃ concentrations for periods extending up to 8 d in duration. Until better preservation techniques have been established, continued immediate (ship-board) analysis of these nutrients is recommended.

Acknowledgments

We acknowledge the Technical Operations Division for sample collection and the staff of the Water Quality Branch Ship Support Laboratory, especially Ms M. Gregory, for excellent analytical work. Special thanks go to R. Stevens for his critical review of this report.

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